

(12) PATENT		(11) Application No. AU 19921155 C	
(19) AUSTRALIAN PATENT OFFICE		(10) Patent No. 746067	
<p>(54) Title Preparation of a lipid blend and a phospholipid suspension containing the lipid blend</p>			
<p>(51)⁶ International Patent Classification(s) A61K 049/00</p>			
(21) Application No: 19921155		(22) Application Date: 1999 . 01 . 14	
(87) WIPO No: WO99/36104			
(30) Priority Data			
(31) Number	(32) Date	(33) Country	
60/071332	1998 . 01 . 14	US	
(43) Publication Date :		1999 . 08 . 02	
(43) Publication Journal Date :		1999 . 10 . 07	
(44) Accepted Journal Date :		2002 . 04 . 11	
(44) Amended Journal Date :		2003 . 06 . 05	
(71) Applicant(s) Du Pont Pharmaceuticals Company			
(72) Inventor(s) Poh K. Hui: John E. Bishop: Eleodoro S. Madrigal Jr.			
(74) Agent/Attorney CALLINAN LAWRIE, Private Bag 7, KEW VIC 3101			
(56) Related Art WO 97/40858 US 5174930			

BEST AVAILABLE COPY

21155/99

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 49/00		A2	(11) International Publication Number: WO 99/36104
			(43) International Publication Date: 22 July 1999 (22.07.99)
(21) International Application Number: PCT/US99/00747		(81) Designated States: AU, BR, CA, CN, CZ, EE, HU, IL, JP, KR, LT, LV, MX, NO, NZ, PL, RO, SG, SI, SK, UA, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 14 January 1999 (14.01.99)		Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(30) Priority Data: 60/071,332 14 January 1998 (14.01.98) US			
(71) Applicant: DU PONT PHARMACEUTICALS COMPANY [US/US]: 974 Centre Road, WR-1ST18, Wilmington, DE 19807 (US).			
(72) Inventors: HUI, Poh, K.; 45 Wareland Road, Wellesley, MA 02181 (US). BISHOP, John, E.; 878 Townsend Road, Groton, MA 01450 (US). MADRIGAL, Eleodoro, S., Jr.; Apartment 12, 14B Strawberry Hill Road, Acton, MA 01720 (US).			
(74) Agent: O'BRIEN, Maureen, P.; Du Pont Pharmaceuticals Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).			

IP AUSTRALIA
0 2 AUG 1999
RECEIVED

(54) Title: PREPARATION OF A LIPID BLEND AND A PHOSPHOLIPID SUSPENSION CONTAINING THE LIPID BLEND

(57) Abstract

The present invention describes processes for the preparation of a lipid blend and a uniform filterable phospholipid suspension containing the lipid blend, such suspension being useful as an ultrasound contrast agent.

TitlePREPARATION OF A LIPID BLEND AND A PHOSPHOLIPID SUSPENSION
CONTAINING THE LIPID BLEND

5

Field of the Invention

The present invention relates generally to processes for the preparation of a lipid blend and a uniform filterable phospholipid suspension containing the lipid blend, such suspension being useful as an ultrasound contrast agent.

Background of the Invention

Manufacturing of a phospholipid contrast agent can be divided into the following steps: (1) preparation of lipid blend; (2) compounding the bulk solution, which involves the hydration and dispersion of the lipid blend in an essentially aqueous medium to produce a lipid suspension; (3) filtration of the bulk solution through a sterilizing filter(s) to render the suspension free of microbial contaminants; (4) dispensing the sterile suspension into individual vials in a controlled aseptic area; (5) loading the dispensed vials into a lyophilizer chamber to replace the vial headspace gas with perfluoropropane gas (PFP); (6) transferring the sealed vials after gas exchange to an autoclave for terminal sterilization. There are three major obstacles in this process: (1) uniformity of the lipid blend; (2) hydration of the lipid blend; (3) uniformity and particle size of the suspension; and, (4) sterile filtration of the suspension through a sterilizing filter(s).

Phospholipid blends are typically produced by dissolving or suspending the required lipids in an appropriate aqueous or non-aqueous solvent system, and then reducing the volume either by lyophilization or distillation. Ideally, this process produces blended solids with high content uniformity and purity. However, while working well on a small, laboratory scale, this simple approach is frequently problematic upon scale-up to

production-size quantities. Difficulties include: (1) maintaining content uniformity during the solvent removal step (due to differential solubilities); (2) maintaining purity (frequently a problem when water is used due to hydrolytic side-reactions); (3) enhancing purity; (4) minimizing solvent volume; and (5) recovery of the final solids (e.g., it is not practical to scrape solids out of a large reactor).

After manufacture of a lipid blend, final compounding typically involves introduction of the blend into an aqueous medium. Since phospholipids are hydrophobic and are not readily soluble in water, adding phospholipids or a lipid blend directly into an aqueous solution causes the lipid powder to aggregate forming clumps that are very difficult to disperse. Thus, the hydration process cannot be controlled within a reasonable process time. Direct hydration of phospholipids or a lipid blend in an aqueous medium produces a cloudy suspension with particles ranging from 0.6 μm to to 100 μm . Due to relatively large particle size distribution, the suspension cannot be filtered at ambient temperature when the suspension solution temperature is below the gel-to-liquid crystal phase transition temperatures of lipids. The lipids would accumulate in the filters causing a restriction in the flow rate, and in most cases, the filters would be completely blocked shortly after. Further reduction in the suspension particle size cannot be achieved through a conventional batching process, even after extended mixing (e.g., 6 hours) at elevated temperatures (e.g., 40°C to 80°C) with a commonly used marine propeller.

Although filtration at elevated temperatures, i.e., at above the phase transition temperatures of lipids, is possible, a significant amount of larger lipid particles would still be excluded when a normal filtering pressure is used. In turn, concentrations of the sterile filtrate would have variable lipid content from batch to batch depending on how the lipids are initially hydrated which is in turn

determined by the physical characteristics, e.g., morphology, of the starting materials.

The process of directly hydrating the lipids or lipid blend to produce a uniform suspension and filtration of the suspension through a sterilization filter(s) can be difficult and costly to be scaled-up to any reasonable commercial scale, e.g., >20L.

Thus, the presently claimed processes for manufacture of a lipid blend and the subsequent phospholipid suspension are aimed at solving the above issues by providing a practical process that can be easily scaled and adopted to various manufacturing facilities without extensive modification or customization of existing equipment.

Summary of the Invention

Accordingly, one aspect of the present invention is to provide a novel process for preparing a lipid blend.

Another aspect of the present invention is to provide a novel process for preparing a phospholipid suspension from the lipid blend.

These and other aspects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that dissolving a lipid blend in a suitable non-aqueous solvent prior to introduction of an aqueous solution allows for production of a phospholipid suspension.



Detailed Description of the Invention

Thus, in a first embodiment, the present invention provides a process for preparing a phospholipid suspension, comprising:

- (a) contacting at least two lipids with a first non-aqueous solvent to form
5 a solution;
- (b) concentrating the solution to a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
- (d) concentrating the solution of step (c) to form a lipid blend;
- 10 (e) contacting the lipid blend with a third non-aqueous solvent, whereby the lipid blend substantially dissolves in the third non-aqueous solvent to form a solution; and,
- (f) without removing the third non-aqueous solvent, contacting the solution from step (e) with an aqueous solution to form a phospholipid suspension.

15

In a preferred embodiment, the non-aqueous solvent of step (e) is selected from the group consisting of propylene glycol, ethylene glycol, and polyethylene glycol 300.

20

In a more preferred embodiment, the non-aqueous solvent of step (e) is propylene glycol.

In another preferred embodiment, the lipid blend, comprises:

- (a) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;
- (b) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic, mono sodium salt; and,
- (c) *N*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-
25 glycero-3-phosphatidylethanolamine, mono sodium salt.



In another preferred embodiment, in step (1), the non-aqueous solvent of step (e) is heated to a temperature of about 30 to 70°C prior to contacting with the lipid blend.

5 In another more preferred embodiment, the non-aqueous solvent of step (e) is heated to a temperature of about 50 to 55°C prior to contacting with the lipid blend.

In another preferred embodiment, the ratio of lipid blend to non-aqueous solvent of step (e) is from about 5 mg of lipid blend per mL of non-aqueous solvent
10 to about 15 mg/mL.

In another more preferred embodiment, the ratio of lipid blend to non-aqueous solvent of step (e) is about 10 mg/mL

In another preferred embodiment, in step (f), the aqueous solution is selected from the group consisting of water, saline, a saline/glycerin mixture, and a
15 saline/glycerin/non-aqueous solvent mixture.

In another more preferred embodiment, the aqueous solution is a saline and glycerin mixture.

In another more preferred embodiment, the aqueous solution is a saline, glycerin, and propylene glycol mixture.

20 In another more preferred embodiment, 6.8 mg/mL of sodium chloride are present, 0.1 mL/mL of glycerin are present, 0.1 mL/mL of propylene glycol are present, and about 0.75 to 1.0 mg/mL of the lipid blend are present.

In an even more preferred embodiment, 0.75 mg/mL of lipid blend are present.



In another more preferred embodiment, 1.0 mg/mL of lipid blend are present.

In another preferred embodiment, in step (f), the aqueous solution is heated to a temperature of about 45 to 60°C prior to contacting with the solution from step 5 (e).

In another more preferred embodiment, the aqueous solution is heated to a temperature of about 50 to 55°C prior to contacting with the solution from step (e).

In another preferred embodiment, the process further comprises:

(g) heating the lipid suspension from step (f) to a temperature about 10 equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the suspension.

13
14
15

In another more preferred embodiment, in step (g), the lipid suspension is heated to a temperature of at least about 67°C.

In another more preferred embodiment, the process further comprises:

16
17
18
19
20

(h) filtering the lipid suspension through a sterilizing filter.

In another even more preferred embodiment, in step (h), the filtration is performed using two sterilizing filter cartridges.

In a further preferred embodiment, in step (h), the sterilizing filter cartridges are at a temperature of from about 70 to 80°C.

In another further preferred embodiment, in step (h), 0.2µm hydrophilic filters are used.

In another even more preferred embodiment, the process further 25 comprises:



(i) dispensing the filtered solution from step (h) into a vial.

In another further preferred embodiment, the process further comprises:

(j) exchanging the headspace gas of the vial from step (j) with a perfluorocarbon gas.

5

In another even further preferred embodiment, the perfluorocarbon gas is perfluoropropane.

In another even further preferred embodiment, exchange of headspace gas is performed using a lyophilizing chamber.

10 In another even further preferred embodiment, the process further comprises:

(k) sterilizing the vial from step (j).

15

In a still further preferred embodiment, in step (k), the vial is sterilized at about 126-130°C for 1 to 10 minutes.

20

In another preferred embodiment, in step (a), the first non-aqueous solvent is a mixture of methanol and toluene.

In another preferred embodiment, in step (c), the second non-aqueous solvent is a methyl *t*-butyl ether.

20 In another preferred embodiment, in step (a), the solution is warmed to a temperature sufficient to complete dissolution of the lipids into the solvent.

In another more preferred embodiment, in step (a), the solution is warmed to about 25 to 75°C.

25 In another preferred embodiment, in step (d), the solids collected are washed with methyl *t*-butyl ether and dried *in vacuo*.



In a second embodiment, the present invention provides a phospholipid suspension whenever prepared by the above process.

In a third embodiment, the present invention provides a process for preparing a phospholipid blend, comprising:

- 5 (a) contacting at least two phospholipids with a first non-aqueous solvent to form a solution;
- (b) concentrating the solution to a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
- 10 (d) concentrating the solution of step (c) to solid phospholipids; and,
- (e) collecting the solid lipids;
- wherein the first non-aqueous solvent is a mixture of methanol and toluene; and wherein the second non-aqueous solvent is methyl t-butyl ether.

- 15 In a fourth embodiment the present invention provides a phospholipid blend whenever prepared by the process as described above.

In a fifth embodiment the present invention provides a process for preparing a solid phospholipid blend, comprising:

- 20 (a) contacting at least two phospholipids with a first non-aqueous solvent to form a solution;
- (b) concentrating the solution into a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent that causes the phospholipids to precipitate as a blend of solid phospholipids; and
- 25 (d) collecting the solid phospholipid blend.
- Preferably, the phospholipids in step (a) are:
- (i) 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine;
- (ii) 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, monosodium salt; and
- (iii) n-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-
- 30 3-phosphatidylethanolamine, monosodium salt.

Preferably the first non-aqueous solvent is a mixture of methanol and toluene and the second non-aqueous solvent is methyl t-butyl ether.

In another preferred aspect the solution of step (a) is warmed to a temperature of from about 25 to about 75°C.



In a preferred aspect the process further comprises washing said blend of solid phospholipids with methyl t-butyl ether and also comprises drying the blend of solid phospholipids in vacuo.

5 In a sixth embodiment, the present invention provides a process for preparing a phospholipid suspension, said process comprising:

- (1) providing a solid phospholipid blend prepared according to the process of the fourth embodiment of the invention;
- (2) contacting said solid phospholipid blend with a non-aqueous polyol solvent
10 whereby the solid phospholipid blend substantially dissolves in said polyol solvent to form a non-aqueous solution; and
- (3) without removing said polyol solvent, contacting the solution from step (2) with an aqueous solution to form a phospholipid suspension.

15 Preferably the polyol solvent is selected from the group consisting of propylene glycol, ethylene glycol, and polyethylene glycol 300. More preferably the polyol solvent is propylene glycol.

In another preferred aspect the process further comprises warming said polyol solvent to a temperature of from about 30°C to about 70°C, more preferably at a temperature of from about 50°C to about 55°C.

20 In yet another preferred aspect the ratio of solid phospholipid blend to polyol solvent is from about 5 mg of solid phospholipid blend per mL of polyol solvent to about 15 mg of solid phospholipid blend per mL of polyol solvent. More preferably the ratio of solid phospholipid blend to polyol solvent is about 10 mg of solid phospholipid blend per mL of polyol solvent.

25 In another preferred aspect the aqueous solution is selected from the group consisting of water, saline, a mixture of saline and glycerin, and a saline, glycerin, and polyol solvent mixture. More preferably the aqueous solution is a mixture of saline and glycerin, or a mixture of saline, glycerin, and propylene glycol.

30 In another preferred aspect the phospholipid suspension comprises 6.8 mg/mL of sodium chloride, 0.1 mL/mL of glycerin, 0.1 mL/mL of propylene glycol, and about 0.75 to 1.0 mg/mL of said solid phospholipid blend, more preferably about 0.75 mg/mL of said solid phospholipid blend, even more preferably about 1.0 mg/mL of said solid phospholipid blend.



In another preferred aspect the phospholipids are:

- (i) 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine;
- (ii) 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, monosodium salt; and
- 5 (iii) n-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt.

In another preferred aspect the phospholipid suspension contains phospholipid particles which are less than 100 nm in diameter, more preferably less than 50 nm in diameter.

- 10 In yet another preferred aspect the process further comprises heating said aqueous solution to a temperature of from about 45°C to about 60°C prior to contacting said aqueous solution with the non-aqueous solution from step (2).

In a more preferred aspect the aqueous solution is heated to a temperature of from about 50°C to about 55°C prior to contacting said aqueous solution with the non-aqueous
15 solution from step (2).

In a further preferred aspect the process further comprises the step of:

- 20 (4) heating said phospholipid suspension to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the phospholipids present in said suspension. Preferably the phospholipid suspension is heated to a temperature of at least about 67°C.

In another preferred aspect the process further comprises the step of:

- 25 (5) filtering said phospholipid suspension through a sterilizing filter. Preferably the filtering is performed using two sterilizing filter cartridges at a temperature of from about 70°C to about 80°C. Preferably, the sterilizing filter cartridges are 0.2 µm sterilizing filter cartridges.

In yet another preferred aspect the process further comprises the step of:

- 30 (6) dispensing the filtered solution from step (5) into a vial. Preferably the vial comprises a headspace containing a first gas, and the process further comprises the step of:
(7) exchanging the first gas in said headspace with a perfluorocarbon gas.

Typically, the perfluorocarbon gas is perfluoropropane and the exchanging of gas is performed using a lyophilizing chamber.

In yet another preferred aspect the process further comprises the step of:

- (8) sterilizing said vial. Preferably the vial is sterilized at about 126°C to about 130°C for 1 to 10 minutes.



In a seventh embodiment, the present invention provides a solid phospholipid blend prepared according to the process of the fifth embodiment described above.

Preferably, the solid phospholipid blend is in the form of a powder.

5 In an eighth embodiment, the present invention provides a phospholipid suspension prepared according to the process of the sixth embodiment described above.

In a ninth embodiment, the present invention provides a vial comprising a phospholipid suspension prepared according to the process of the sixth embodiment described above.

10 Preferably the vial further comprises a headspace comprising a perfluorocarbon gas.

More preferably, the perfluorocarbon gas is perfluoropropane.

Formulation

The present invention is contemplated to be practiced on at least a multigram scale,

15 kilogram scale, multikilogram scale, or industrial scale. Multigram scale, as used herein, is preferably the scale wherein at least one starting material is present in 10 grams or more, more preferably at least 50 grams or more, even more preferably at least 100 grams or more. Multikilogram scale, as used herein, is intended to mean the scale wherein more than one kilogram of at least one

20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100



starting material is used. Industrial scale as used herein is intended to mean a scale which is other than a laboratory scale and which is sufficient to supply product sufficient for either clinical tests or distribution to consumers.

Lipid blend or phospholipid blend, as used herein, is intended to represent
5 two or more lipids which have been blended. The lipid blend is generally in a powder form. Preferably, at least one of the lipids is a phospholipid. Preferably, the lipid blend contains 1,2-dipalmitoyl-*sn*-

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217

glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidic, mono sodium salt (DPPA), and N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt (MPEG5000-DPPE). The amount of each lipid present in the blend will depend on the desired end product. Preferred ratios of each lipid are described in the Examples section. A wide variety of other lipids, like those described in Unger et al, U.S. Patent No. 5,469,854, the contents of which are hereby incorporated by reference, may be used in the present process.

Phospholipid, as used herein, is a fatty substance containing an oily (hydrophobic) hydrocarbon chain(s) with a polar (hydrophilic) phosphoric head group. Phospholipids are amphiphilic. They spontaneously form boundaries and closed vesicles in aqueous media. Phospholipids constitute about 50% of the mass of animal cell plasma membrane.

Preparation of the lipid blend

The lipid blend may be prepared via an aqueous suspension-lyophilization process or an organic solvent dissolution-precipitation process using organic solvents. In the aqueous suspension-lyophilization process, the desired lipids are suspended in water at an elevated temperature and then concentrated by lyophilization. Preferably a dissolution procedure is used.

Step (a):

The organic solvent dissolution-precipitation procedure involves contacting the desired lipids (e.g., DPPA, DPPC, and MPEG5000 DPPE) with a first non-aqueous solvent system. This system is typically a combination of solvents, for example $\text{CHCl}_3/\text{MeOH}$, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, and toluene/MeOH. Preferably, the first non-aqueous solvent is a mixture of toluene and methanol. It may be desirable to warm the lipid solution to a temperature sufficient to achieve complete dissolution. Such a temperature is preferably about 25 to 75°C, more preferably about 35 to 65°C.

After dissolution, it may be desired to remove undissolved foreign matter by hot-filtration or cooling to room temperature and then filtering. Known methods of filtration may be used (e.g., gravity filtration, vacuum
5 filtration, or pressure filtration).

Step (b):

The solution is then concentrated to a thick gel/semisolid. Concentration is preferably done by vacuum
10 distillation. Other methods of concentrating the solution, such as rotary evaporation, may also be used. The temperature of this step is preferably about 20 to 60°C, more preferably 30 to 50°C.

15 Step (c):

The thick gel/semisolid is then dispersed in a second non-aqueous solvent. The mixture is slurried, preferably near ambient temperature (e.g., 15-30°C). Useful second non-aqueous solvents are those that cause the lipids to
20 precipitate from the filtered solution. The second non-aqueous solvent is preferably methyl t-butyl ether (MTBE). Other ethers and alcohols may be used.

Step (d):

25 The solids produced upon addition of the second non-aqueous solvent are then collected. Preferably the collected solids are washed with another portion of the second non-aqueous solvent (e.g., MTBE). Collection may be performed via vacuum filtration or centrifugation,
30 preferably at ambient temperature. After collection, it is preferred that the solids are dried in vacuo at a temperature of about 20-60°C.

For the following reasons, the organic solvent
35 dissolution-precipitation process is preferred over the aqueous suspension/lyophilization process:

(1) Because the lipids are quite soluble in toluene/methanol, solvent volumes are significantly reduced (relative to the aqueous procedure).

(2) Because of this increased solubility, the process temperature is also lower relative to the aqueous procedure, thereby avoiding the hydrolytic instability of fatty acid esters.

(3) When cooled back to room temperature, the toluene/methanol solution of lipids remains homogeneous, allowing a room temperature filtration to remove solid foreign matter.

(4) The MTBE precipitation allows quick and easy isolation of Lipid Blend solids. With the aqueous process, a time-consuming lyophilization process is used to isolate material.

(5) The MTBE precipitation also allows for the removal of any MTBE-soluble impurities, which go into the filtrate waste-stream. This potential for impurity removal is not realized when a solution is directly concentrated or lyophilized to a solid.

(6) The present process affords uniform solids.

Preparation of the lipid suspension

Step (1):

In step one, a lipid blend is contacted with a non-aqueous solvent, whereby the lipid blend substantially dissolves in the non-aqueous solvent. Alternatively, the individual lipids may be contacted with the non-aqueous solvent sequentially in the order: DPPC; DPPA, and MPEG5000-DPPE; DPPC, MPEG5000-DPPE, and DPPA; MPEG5000-DPPE, DPPA, and DPPC; or MPEG5000-DPPE, DPPC, and DPPA. The DPPA, being the least soluble and least abundant of the lipids is not added first. Adding one of the other lipids prior to or concurrently with adding the DPPA facilitates dissolution of the DPPA. In another alternative, the individual lipids can be combined in their solid forms and the combination of the solids contacted with the non-aqueous solvent.

Substantial dissolution is generally indicated when the mixture of lipid blend and non-aqueous solvent becomes clear. As noted previously, phospholipids are generally not water soluble. Thus, direct introduction of a blend of
5 phospholipid blend into an aqueous environment causes the lipid blend to aggregate forming clumps that are very difficult to disperse. The present invention overcomes this limitation by dissolving the lipid blend in a non-aqueous solvent prior to introduction of the aqueous solution. This
10 allows one to evenly disperse the lipid blend into a liquid. The liquid dispersion can then be introduced into a desired aqueous environment.

Non-aqueous is intended to mean a solvent or mixture of solvents wherein the amount of water present is sufficiently
15 low as to not impede dissolution of the lipid blend. The amount of non-aqueous solvent required will depend on the solubility of the lipid blend and also the final desired concentration of each component. As one of ordinary skill would appreciate, the level of water present in the non-
20 aqueous solvent, which may be tolerated will vary based on the water solubilities of the individual lipids in the lipid blend. The more water soluble the individual phospholipids, the more water which may be present in step (1). Preferably, propylene glycol is used as the non-aqueous
25 solvent. However, other members of the polyol family, such as ethylene glycol, and polyethylene glycol 300 may be used.

Mechanically mixing the lipid blend and non-aqueous solvent may be necessary to achieve complete dissolution. One of ordinary skill in the art will recognize that a
30 variety of ways of mixing are available. It is preferred that a high shear homogenizer is used.

One of ordinary skill in the art would recognize that raising the temperature of the solvent should aid in dissolution of the lipid blend. The temperature at which
35 step (1) may be performed can range from ambient to the boiling point of the chosen solvent. Preferably the temperature is from about 30 to about 70°C, more preferably about 45 to about 60°C, and even more preferably about 50,

51, 52, 53, 54, or 55°C. When ethylene glycol or polyethylene glycol 300 is used, it is preferred that the temperature be from about 50 to about 60°C and more preferably about 55°C. Maintaining the solution at an elevated temperature should reduce solution viscosity and ease formulation preparation.

A preferred procedure for dissolving the lipid blend is as follows: (a) Add propylene glycol to an appropriate weighing container. (b) Warm the propylene glycol to about 40-80°C in a heating bath. (c) Weigh the lipid blend into a separate container. (d) When the propylene glycol has reached the desired temperature range, transfer the solution into the container containing the lipid blend. (e) Place the container back into the heating bath until the solution is clear. (f) Mechanically mix the Lipid Blend/Propylene Glycol solution to further assure complete dissolution and uniform dispersion of the lipid blend.

The ratio of lipid blend to non-aqueous solvent will, of course, be limited by the solubility of the lipid blend. This ratio will also be influenced by the desired amount of lipid blend in the final formulation. Preferably, the ratio is from about 1 mg of lipid blend per mL of solvent (mg/mL) to about 100 mg/mL. More preferably, the lipid blend is present in about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mg/mL. Even more preferably, the lipid blend is present in about 10 mg/mL.

Step (2):

The second step involves contacting the solution from step (1) with an aqueous solution to form a lipid suspension. The aqueous solution can be water, saline, a saline/glycerin mixture or a saline/glycerin/non-aqueous solvent mixture. Non-aqueous solvent is as defined previously, preferably propylene glycol. Suspension, as used herein, is intended to indicate a dispersion in which insoluble particles are dispersed in a liquid medium.

Once complete dissolution of the lipid blend has been achieved (step (1)), the resulting solution can then be

introduced to an aqueous solution. The aqueous solution may contain one or more components selected from sodium chloride, glycerin, and a non-aqueous solvent. Preferably the aqueous solution contains glycerin and sodium chloride.

- 5 Preferably, a sufficient amount of propylene glycol is present in the aqueous solution, prior to addition of the solution from step 1, in order to achieve the final desired concentration of propylene glycol.

- The order of addition of desired components is not
10 expected to seriously impact the resulting lipid suspension. However, it is preferred that the lipid-blend solution is added to water, which may already contain the above-noted additional components. Additional desired components can then be added. It is more preferred that the lipid-blend
15 solution is added a solution of water and sodium chloride (i.e., saline). It is further preferred that the lipid-blend solution is added a solution of water, sodium chloride, and glycerin. It is still further preferred that the lipid-blend solution is added a solution of water,
20 sodium chloride, glycerin, and propylene glycol.

- It is preferred that 6.8 mg of NaCl are present per mL of formulation. Preferably, 0.1 mL of Glycerin per mL of formulation is present. A final concentration of 0.1 mL of Propylene Glycol per mL of formulation is preferred. The
25 final pH of the formulation is preferably about 5.5-7.0. The lipid blend is preferably present in an amount of 0.75-1.0 mg/mL of formulation.

- The temperature of the aqueous solution can range from ambient to 70°C. Preferably, the temperature is about 45 to
30 60°C, with 50, 51, 52, 53, 54, or 55 being even more preferred. In order to obtain complete dissolution, the mixture will need to be agitated, preferably stirred. Also, the pH of the solution may need to be adjusted, depending on the desired final formulation. Either acid (e.g., HCl) or
35 base (e.g., NaOH) can be added to make such an adjustment.

The lipid suspension will contain liquid particles of varying sizes. One of the benefits of the present invention is the ability to consistently obtain small particles of a

nearly uniform size. Thus, it is preferred that the majority of particles obtained are less than 100 nm in diameter, more preferable less than 50 nm.

A preferred procedure for dissolving the lipid blend is as follows: (a) Add Water for Injection (WFI) into a compounding vessel. (b) Start mixing and ensure temperature is from 50-55°C. (c) Add sodium chloride to the compounding vessel. Wait until the solid has completely dissolved before proceeding to the next step. (d) Add glycerin to the compounding vessel. Allow sufficient time for complete mixing. (e) Add the remaining Propylene Glycol that is not in the Lipid Blend/Propylene Glycol solution. Allow time for thorough mixing. (f) Reduce mixing rate to reduce turbulence in the compounding vessel. (g) Add the Lipid Blend/Propylene Glycol solution to the compounding vessel. (h) Readjust mixing to original rate. (i) Add additional WFI if necessary. (j) Continue to mix for approximately 25 minutes and assure complete mixing. (k) Verify and adjust the solution to target pH.

Step(3):

Step three involves heating the lipid suspension obtained from step (2) to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the solution.

One of the objects of this step is to provide a filterable suspension. A solution/suspension is considered filterable if there is no significant reduction in flow rate within a normal process, and there is no significant increase in the pressure drop in the filtration system.

Experimental data indicates that the lipids in the formulation should be beyond their gel to liquid crystalline phase transition in order to simplify sterile filtration. When the lipids are below the phase transition temperature, the suspension particles are rigid. However, when they are above their respective gel-liquid crystal phase transition temperatures, they are in a more loosely organized configuration and thus, more easily filtered.

DPPC and DPPA show phase transitions of 41°C and 67°C respectively. MPEG5000-DPPE is soluble in water, therefore it does not exhibit a gel-liquid crystal phase transition which is characteristic of most hydrated lipid suspensions. 5 Because the lipids in the preferred formulation all exhibit different gel to liquid phase transitions, the highest phase transition temperature, 67°C, is preferably used to filter the solution. By maintaining temperature at or beyond 67°C, all the lipids are beyond their respective phase transition, 10 assuring the loose configuration while passing through the filters.

Heating may be achieved by jacketing the compounding vessel with a heat exchanging coil. Hot water/steam from a controlled source, e.g., a hot water bath, or a water 15 heater, would deliver sufficient heat to maintain the compounding solution at a set temperature. Other heat sources known to those of skill in the art could also be used.

20 Step (4):

Step four is performed by filtering the lipid suspension through a sterilizing filter. The purpose behind this step being to provide a substantially bacteria-free suspension. A filtrate is considered substantially 25 bacteria-free when the probability of the filtrate to contain at least one colony forming microorganism is less than 10^{-6} .

Filtration is preferably done using sterilizing filter cartridges. Also, a means of forcing the solution through 30 the filters may be required (e.g., pumping or pressurizing). Since the solution being filtered needs to be maintained at a temperature at or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the solution, the filtration should be performed 35 at about this same temperature. In order to accomplish this, the filter (e.g., sterilizing filter cartridges) are preferably enclosed in jacketed filter housings which are continuously heated, e.g., by a hot water stream from a

temperature controlled water bath, to ensure that the suspension is above the lipid phase transition temperatures. The temperature of the sterilizing filter is preferably from 50 to 100°C, more preferably from 60 to 90°C, and even more preferably 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80°C.

One or more sterilizing filters may be used to filter the suspension. The required number will be based on their effectiveness at removing bacteria. It is preferred that two filters are used. The size of the filter pores will be limited by the need to provide a bacteria-free suspension. Preferably, 0.2µm hydrophilic filters are used.

A bulk solution of the preferred formulation was continuously filtered through two 0.2µm hydrophilic filters for up to 3 hours at a rate of approximately 1 liter per minute (1 L/min.), i.e., passing a total of 180 liters of the suspension solution through the filters. The experimental results shows that there is no apparent blockage of filters. Lipid assays indicates that there is no measurable loss during the filtration process (due to accumulation in the filter medium).

A bulk solution of the preferred formulation was compounded at 40°C-80°C, and the suspension was cooled to ambient temperature prior to sterile filtration. No apparent clogging of the filters were observed indicating the suspension particle size distribution is well below 0.2µm of the filter pore size. It is desirable to use heat during filtration in order to ensure maximum recover of the lipid blend in the sterile filtrate (i.e., to minimize potential retention of lipid particles in the filter medium).

A preferred procedure for filtering the lipid suspension is as follows: (a) Assure all jacketed filters are at 70°C - 80°C. (b) Assure all valves in the filtration unit are closed. (c) Connect filtration inlet hose to the outlet of the compounding vessel. (d) Open valves to allow solution to pass through the filters. (e) Flush three liters of solution through the filters before collecting filtrate. (f) Continue filtration until complete.

Step (5):

Dispensing the filtered solution into a vial completes step five. Preferably, this step is performed in a controlled aseptic area. One of ordinary skill in the art would recognize that the vial selected and amount of suspension delivered to the vial would depend on the end use considered for the lipid suspension. Dispensing can be achieved via a variety of methods, including pipette, hand-held syringe dispenser (e.g., Filamatic® syringe dispensing machine), or industrial auto dispensing machine (e.g., Cozzoli or TL auto filling machine).

Step (6):

Step six is performed by exchanging the headspace gas of the vials from step five with a perfluorocarbon gas. A preferred method of exchange is to load the dispensed vials into a lyophilizer chamber and replace the vial headspace gas with a perfluorocarbon gas. A preferred gas is perfluoropropane (PFP). Other methods of headspace gas exchange known to those of skill in the art may be employed.

The vials are sealed at the completion of the vial headspace gas exchange cycle. When the lyophilizer chamber pressure is brought back to atmospheric pressure by charging into the chamber with PFP. Vial stoppers are seated to seal the vials.

Step (7):

Step seven involves terminally sterilizing a vial after step six. One method of terminal sterilization is through the use of an autoclave. Also, the sealed vials can be terminally sterilized in a steam sterilizer to further enhance the sterility assurance of the product. Care must be taken in the sterilization process as some degradation of lipids may be observed as a result of autoclaving. Preferably, the vial is sterilized at about 126-130°C for 1 to 10 minutes.

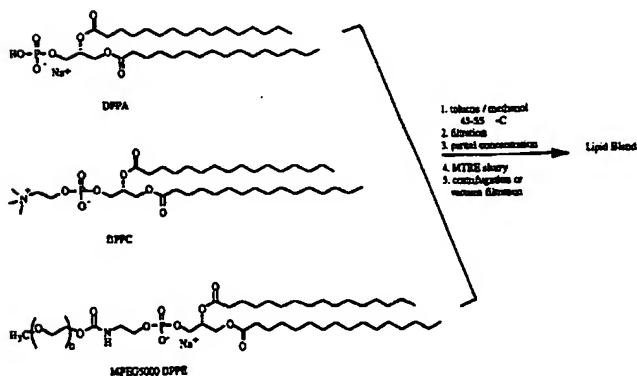
Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

5

EXAMPLES**Table 1: Lipid Blend Target Composition**

Lipid Name	Common Name	Wt %	Mole %
DPPA	1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, monosodium salt	6.0	10
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine	53.5	82
MPEG5000 DPPE	N - (methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt	40.5	8

10

Lipid Blend Manufacturing Procedure

A flask is charged with toluene (3.3 L), methanol (1.2 L), DPPA (59.6 g), DPPC (535 g), and MPEG5000 DPPE (405 g).

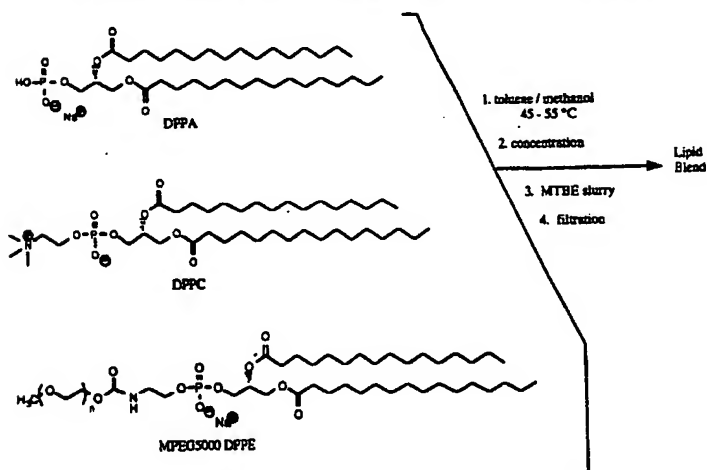
20

After rinsing solid contact surfaces with 0.9 L methanol, the slurry is warmed to 45-55°C until dissolution is complete.

The solution is filtered and then concentrated in vacuo at 35 - 45° C to a thick gel. Methyl t-butyl ether (MTBE, 5.4 L) is added and the mixture is slurried at 15-30°C. White solids are collected by centrifugation or vacuum filtration, and washed with MTBE (0.9 L). The solids are then placed in a vacuum oven and dried to constant weight at 40-50°C. The dried Lipid Blend is transferred to a bottle and stored at -15 to -25 °C.

In another embodiment of the lipid blend manufacturing procedure of the present invention, the following procedure may also be used.

Alternative Lipid Blend Manufacturing Procedure



20

Phospholipid quantities were adjusted for purity based on a "Use As" value from the certificates of analysis. The

batch size (combined phospholipid weight) of this experiment was 2 kg.

A rotary evaporation flask is charged sequentially with toluene (3,300 mL), methanol (1,200 mL), DPPA (122.9 g; 5 corrected for "use as" purity of 97.0 %), DPPC (1,098.5 g total; 500.8 g from a lot with 98.4 % "use as" purity and 597.7 g from a lot with 96.7 % "use as" purity), and MPEG5000 DPPE (815.7 g; corrected for "use as" purity of 99.3 %). After rinsing residual solids into the flask with 10 methanol (900 mL), the flask is placed on a rotary evaporator (no vacuum) and the slurry is warmed to between 45 and 55 °C (external). After dissolution is complete, the external temperature is reduced to between 35 and 45 °C, a vacuum is applied, and the solution is concentrated to a 15 white semi-solid. The flask is removed from the evaporator and solids are broken up with a spatula. The flask is reapplied to the evaporator and concentration is continued. After reaching the endpoint (final vacuum pressure = 20 mbar; white, granular, chunky solid), MTBE (5,400 mL) is 20 added through the rotary evaporator's addition tube, the vacuum is discontinued, and the mixture is slurried for 15 to 45 min at 15 to 30 °C. Solids are isolated by either centrifugal or vacuum filtration, rinsed with MTBE (3,800 mL), and dried to constant weight in a vacuum oven (40 to 50 25 °C). Prior to transferring to polyethylene bottles with polypropylene caps, solids are delumped through a screen (0.079 inch mesh), affording 1,966.7 g (98 %) of lipid blend (SG896) as a white solid.

30 The preferred lipid suspension contains:
1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic, mono
sodium salt (DPPA);
1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine
(DPPC);
35 N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-
dipalmitoyl-*sn*-glycero-3-

- phosphatidylethanolamine, monosodium salt
(MPEG5000-DPPE);
Propylene Glycol, USP;
Glycerin, USP;
5 Sodium Chloride, USP; and,
Water for Injection, USP.

Table 2: Preferred Contrast Agent Formulations

Component	A*	B*
NaCl, USP	6.8 mg/mL	6.8 mg/mL
Glycerin, USP	0.1 mL/mL	0.1 mL/mL
Propylene Glycol, USP	0.1 mL/mL	0.1 mL/mL
Lipid Blend**	1 mg/mL	0.75 mg/mL
Perfluoropropane	> 65%	> 65%
pH	6.0 - 7.0	6.0 - 7.0

- 10 *Formulation A has 1 mg/mL lipid blend. Formulation B has a lipid blend concentration of 0.75 mg/mL.
**The lipid blend is consist of 53.5 wt.% of DPPC, 6.0 wt.% of DPPA and 40.5 wt.% of MPEG5000-DPPE.

15

Table 3: Preferred Container and Closure

Component	Type
Vial	Wheaton 2802, B33BA, 2cc, 13mm, Type I, flint tubing vial
Stopper	West V50 4416/50, 13mm, gray butyl lyo, siliconized stoppers
Seal	West 3766, white 13mm, flip-off aluminum seals

The finished product fill volume can be from 1.0-2.0 mL/vial.

- 20 In the preparation of the preferred formulation, when the lipid blend is directly hydrated with the aqueous matrix solution containing water for injection, sodium chloride,

glycerin and propylene glycol, the filtrates have less lipids as compared to the pre-filtration bulk solution. The loss of lipids varies from 12% to 48%. These results demonstrate that the sterile filtration process is not effectively controlled, and therefore, the final product lipid content is highly variable.

In contrast, using the presently described process, assay results of the lipids in show full recovery of lipids during the filtration process. Variability of assay results around the theoretical targets is within normal assay method variability. Particle size distribution by number, by volume and by reflective intensity of a suspension prepared by first solubilizing lipid blend in propylene glycol indicate that the majority of the particles are less than 50 nm in the pre-filtered bulk solution at 55°C as well at 70°C. The particle distribution profile does not change after filtration.

UTILITY SECTION

The presently claimed process is useful for preparing ultrasound contrast agents. Such agents should be useful for a variety of imaging applications, including enhancing contrast in echocardiographic and radiologic ultrasound images.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification, they are to be interpreted as specifying the presence of the stated features, integers, steps or components referred to, but not to preclude the presence or addition of one or more other feature, integer, step,
5 component or group thereof.



The claims defining the invention are as follows:

1. A process for preparing a phospholipid suspension, comprising:
 - (a) contacting at least two lipids with a first non-aqueous solvent to form a solution;
 - 5 (b) concentrating the solution to a thick gel;
 - (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
 - (d) concentrating the solution of step (c) to form a lipid blend;
 - (e) contacting the lipid blend with a third non-aqueous solvent, whereby
 - 10 the lipid blend substantially dissolves in the third non-aqueous solvent to form a solution; and,
 - (f) without removing the third non-aqueous solvent, contacting the solution from step (e) with an aqueous solution to form a phospholipid suspension.
2. A process according to claim 1, wherein the non-aqueous solvent of
- 15 step (e) is selected from the group consisting of propylene glycol, ethylene glycol, and polyethylene glycol 300.
3. A process according to claim 2, wherein the non-aqueous solvent of step (e) is propylene glycol.
4. A process according to any one of claims 1 to 3, wherein the lipid
- 20 blend comprises:
 - (a) 1, 2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;
 - (b) 1, 2- dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt; and
 - (c) N-(methoxypolyethylene glycol 5000 carbamoyl) -1, 2- dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, mono sodium salt.
- 25 5. A process according to claim 2, wherein the non-aqueous solvent of step (e) is heated to a temperature of about 30 to 70°C prior to contacting with the lipid blend.
6. A process according to claim 5, wherein the non-aqueous solvent of step (e) is heated to a temperature of about 50 to 55°C prior to contacting with the
- 30 lipid blend.
7. A process according to claim 2, wherein the ratio of lipid blend to non-aqueous solvent of step (e) is from about 5 mg of lipid blend per mL of non-aqueous solvent to about 15 mg/mL.



8. A process according to claim 7, wherein the ratio of lipid blend to non-aqueous solvent of step (e) is about 10 mg/mL.

9. A process according to any one of claims 1 to 8, wherein in step (f), the aqueous solution is selected from the group consisting of water, saline, a saline/glycerin mixture, and a saline/glycerin/non-aqueous solvent mixture.

10. A process according to claim 9, wherein the aqueous solution is a saline and glycerin mixture.

11. A process according to claim 9, wherein the aqueous solution is a saline, glycerine, and propylene glycol mixture.

12. A process according to claim 11, wherein 6.8 mg/mL of sodium chloride are present, 0.1 mL/mL of glycerin are present, 0.1 mL/mL of propylene glycol are present, and about 0.75 to 1.0 mg/mL of the lipid blend are present.

13. A process according to claim 12, wherein 0.75 mg/mL of lipid blend are present.

14. A process according to claim 12, wherein 1.0 mg/mL of lipid blend are present.

15. A process according to any one of claims 1 to 4, wherein in step (f), the aqueous solution is heated to a temperature of about 45 to 60°C prior to contacting with the solution from step (e).

16. A process according to claim 15, wherein the aqueous solution is heated to a temperature of about 50 to 55°C prior to contacting with the solution from step (e).

17. A process according to any one of claims 1 to 16, wherein the process further comprises:

(g) heating the lipid suspension from step (f) to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the suspension.

18. A process according to claim 17, wherein in step (g), the lipid suspension is heated to a temperature of at least about 67°C.

19. A process according to claim 17, wherein the process further comprises:

(h) filtering the lipid suspension through a sterilizing filter.

20. A process according to claim 19, wherein in step (h), the filtration is performed using two sterilizing filter cartridges.



21. A process according to claim 20, wherein in step (h), the sterilizing filter cartridges are at a temperature of from about 70 to 80°C.

22. A process according to claim 21, wherein in step (h), 0.2µm hydrophilic filters are used.

5 23. A process according to claim 19, wherein the process further comprises:

(i) dispensing the filtered solution from step (h) into a vial.

24. A process according to claim 23, wherein the process further comprises:

10 (j) exchanging the headspace gas of the vial from step (i) with a perfluorocarbon gas

25. A process according to claim 24, wherein the perfluorocarbon gas is perfluoropropane.

15 26. A process according to claim 25, wherein exchange of headspace gas is performed using a lyophilizing chamber.

27. A process according to claim 24, wherein the process further comprises:

(k) sterilizing the vial from step (j).

20 28. A process according to claim 27, wherein in step (k), the vial is sterilized at about 126-130°C for 1 to 10 minutes.

29. A process according to any one of claims 1 to 28, wherein in step (a), the first non-aqueous solvent is a mixture of methanol and toluene.

30. A process according to any one of claims 1 to 29, wherein in step (c), the second non-aqueous solvent is a methyl *t*-butyl ether.

25 31. A process according to any one of claims 1 to 30, wherein in step (a), the solution is warmed to a temperature sufficient to complete dissolution of the lipids into the solvent.

32. A process according to claim 31, wherein in step (a), the solution is warmed to about 25 to 75°C.

30 33. A process according to any one of claims 1 to 32, wherein in step (d), the solids collected are washed with methyl *t*-butyl ether and dried in *vacuo*.

34. A process for preparing a phospholipid blend, comprising:

(a) contacting at least two phospholipids with a first non-aqueous solvent to form a solution;



- (b) concentrating the solution to a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
- (d) concentrating the solution of step (c) to solid phospholipids; and,
- (e) collecting the solid lipids;

wherein the first non-aqueous solvent is a mixture of methanol and toluene; and wherein the second non-aqueous solvent is methyl t-butyl ether.

35. A process of any one of claims 1 to 33 for preparing a phospholipid suspension which process is substantially as herein described with reference to any one of the Examples.

36. A phospholipid suspension whenever prepared by the process of any one of claims 1 to 33 or claim 35.

37. A process of claim 34 for preparing a phospholipid blend which process is substantially as herein described with reference to any one of the Examples.

38. A phospholipid blend whenever prepared by the process of claim 34 or claim 37.

39. A process for preparing a solid phospholipid blend, comprising:

- (a) contacting at least two phospholipids with a first non-aqueous solvent to form a solution;
- (b) concentrating the solution into a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent that causes the phospholipids to precipitate as a blend of solid phospholipids; and
- (d) collecting the solid phospholipid blend.

40. A process according to claim 39, wherein in step (a), the phospholipids are:

- (i) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;
- (ii) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic acid, monosodium salt; and
- (iii) *n*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, monosodium salt.



41. A process according to claim 39 or claim 40, wherein said first non-aqueous solvent is a mixture of methanol and toluene.

42. A process according to any one of claims 39 to 41, wherein said second non-aqueous solvent is methyl t-butyl ether.

43. A process according to any one of claims 39 to 42, wherein the solution of step (a) is warmed to a temperature of from about 25 to about 75°C.

44. A process according to any one of claims 39 to 43, further comprising washing said blend of solid phospholipids with methyl t-butyl ether.

45. A process according to any one of claims 39 to 44, further comprising drying the blend of solid phospholipids *in vacuo*.

46. A process for preparing a phospholipid suspension, said process comprising:
(1) providing a solid phospholipid blend prepared according to the process of any one of claims 39 to 45;
(2) contacting said solid phospholipid blend with a non-aqueous polyol solvent whereby the solid phospholipid blend substantially dissolves in said polyol solvent to form a non-aqueous solution; and
(3) without removing said polyol solvent, contacting the solution from step (2) with an aqueous solution to form a phospholipid suspension.

47. A process according to claim 46, wherein said polyol solvent is selected from the group consisting of propylene glycol, ethylene glycol, and polyethylene glycol 300.

48. A process according to claim 47, wherein said polyol solvent is propylene glycol.



49. A process according to any one of claims 46 to 48, further comprising warming said polyol solvent to a temperature of from about 30°C to about 70°C.

50. A process according to claim 49, comprising warming said polyol solvent to a temperature of from about 50°C to about 55°C.

51. A process according to any one of claims 46 to 50, wherein the ratio of solid phospholipid blend to polyol solvent is from about 5 mg of solid phospholipid blend per mL of polyol solvent to about 15 mg of solid phospholipid blend per mL of polyol solvent.

52. A process according to claim 51, wherein the ratio of solid phospholipid blend to polyol solvent is about 10 mg of solid phospholipid blend per mL of polyol solvent.

53. A process according to any one of claims 46 to 52, wherein said aqueous solution is selected from the group consisting of water, saline, a mixture of saline and glycerin, and a saline, glycerin, and polyol solvent mixture.

54. A process according to claim 53, wherein said aqueous solution is a mixture of saline and glycerin.

55. A process according to claim 53, wherein said aqueous solution is a mixture of saline, glycerin, and propylene glycol.

56. A process according to any one of claims 46 to 55, wherein said phospholipid suspension comprises 6.8 mg/mL of sodium chloride, 0.1 mL/mL of glycerin, 0.1 mL/mL of propylene glycol, and about 0.75 to 1.0 mg/mL of said solid phospholipid blend.

57. A process according to claim 56, containing about 0.75 mg/mL of said solid phospholipid blend.



58. A process according to claim 56, containing about 1.0 mg/mL of said solid phospholipid blend.

59. A process according to any one of claims 46 to 58, wherein the phospholipids are:

- (i) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;
- (ii) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic acid, monosodium salt; and
- (iii) *n*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, monosodium salt.

60. A process according to any one of claims 46 to 55, wherein said phospholipid suspension contains phospholipid particles which are less than 100 nm in diameter.

61. A process according to claim 60, wherein said phospholipid suspension contains phospholipid particles which are less than 50 nm in diameter.

62. A process according to any one of claims 46 to 61, further comprising heating said aqueous solution to a temperature of from about 45°C to about 60°C prior to contacting said aqueous solution with the non-aqueous solution from step (2).

63. A process according to claim 62, wherein said aqueous solution is heated to a temperature of from about 50°C to about 55°C prior to contacting said aqueous solution with the non-aqueous solution from step (2).

64. A process according to any one of claims 46 to 63, further comprising the step of:
(4) heating said phospholipid suspension to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the phospholipids present in said suspension.

65. A process according to claim 64, wherein in step (4) said phospholipid suspension is heated to a temperature of at least about 67°C.



66. A process according to any one of claims 46 to 65, further comprising the step of:

(5) filtering said phospholipid suspension through a sterilizing filter.

5 67. A process according to claim 66, wherein said filtering is performed using two sterilizing filter cartridges.

68. A process according to claim 67, wherein said sterilizing filter cartridges are at a temperature of from about 70°C to about 80°C.

10 69. A process according to claim 68, wherein said sterilizing filter cartridges are 0.2 µm sterilizing filter cartridges.

70. A process according to any one of claims 46 to 69, further comprising the step of:

(6) dispensing the filtered solution from step (5) into a vial.

71. A process according to claim 70, wherein said vial comprises a headspace containing a first gas, and further comprising the step of:

20 (7) exchanging the first gas in said headspace with a perfluorocarbon gas.

72. A process according to claim 71, wherein said perfluorocarbon gas is perfluoropropane.

25 73. A process according to claim 71, wherein said exchanging of gas is performed using a lyophilizing chamber.

74. A process according to any claim 71, further comprising the step of:

(8) sterilizing said vial.

30 75. A process according to claim 74, wherein said vial is sterilized at about 126°C to about 130°C for 1 to 10 minutes.



76. A solid phospholipid blend prepared according to the process of any one of claims 39 to 45.

77. A solid phospholipid blend according to claim 76, wherein said blend is in the form of a powder.

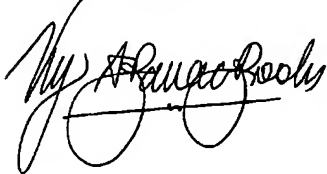
78. A phospholipid suspension prepared according to the process of any one of claims 46 to 75.

79. A vial comprising a phospholipid suspension prepared according to the process of any one of claims 46 to 75.

80. A vial according to claim 79, further comprising a headspace comprising a perfluorocarbon gas.

81. A vial according to claim 80 wherein said perfluorocarbon gas is perfluoropropane.

Dated this 16th day of July, 2002
DUPONT PHARMACEUTICALS COMPANY
By their Patent Attorneys:
CALLINAN LAWRIE



1507/02, aw11353a, 33



(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199921155 B2
(10) Patent No. 746067

(54) Title
Preparation of a lipid blend and a phospholipid suspension containing the lipid blend

(51)⁶ International Patent Classification(s)
A61K 049/00

(21) Application No: 199921155 (22) Application Date: 1999 .01 .14

(87) WIPO No: W099/36104

(30) Priority Data

(31) Number (32) Date (33) Country
60/071332 1998 .01 .14 US

(43) Publication Date: 1999 .08 .02

(43) Publication Journal Date: 1999 .10 .07

(44) Accepted Journal Date: 2002 .04 .11

(71) Applicant(s)
Du Pont Pharmaceuticals Company

(72) Inventor(s)
Poh K. Hui; John E. Bishop; Eleodoro S. Madrigal Jr.

(74) Agent/Attorney
CALLINAN LAWRIE,Private Bag 7,KEW VIC 3101

(56) Related Art
WO 97/40858
US 5174930

21155/99

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 49/00	A2	(11) International Publication Number: WO 99/36104 (43) International Publication Date: 22 July 1999 (22.07.99)
(21) International Application Number: PCT/US99/00747 (22) International Filing Date: 14 January 1999 (14.01.99) (30) Priority Data: 60071,332 14 January 1998 (14.01.98) US (71) Applicant: DU PONT PHARMACEUTICALS COMPANY [US/US]: 974 Centre Road, WR-1ST18, Wilmington, DE 19807 (US). (72) Inventors: HUI, Poh, K.; 45 Wareland Road, Wellesley, MA 02181 (US). BISHOP, John, E.; 878 Townsend Road, Groton, MA 01450 (US). MADRIGAL, Eleodoro, S., Jr.; Apartment 12, 14B Strawberry Hill Road, Acton, MA 01720 (US). (74) Agent: O'BRIEN, Maureen, P.; Du Pont Pharmaceuticals Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).	(81) Designated States: AU, BR, CA, CN, CZ, EE, HU, IL, JP, KR, LT, LV, MX, NO, NZ, PL, RO, SG, SI, SK, UA, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TI, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i> <div data-bbox="941 798 1193 945" style="border: 1px solid black; padding: 5px; text-align: center;"> IP AUSTRALIA 0 2 AUG 1999 RECEIVED </div>	
(54) Title: PREPARATION OF A LIPID BLEND AND A PHOSPHOLIPID SUSPENSION CONTAINING THE LIPID BLEND (57) Abstract The present invention describes processes for the preparation of a lipid blend and a uniform filterable phospholipid suspension containing the lipid blend, such suspension being useful as an ultrasound contrast agent.		

TitlePREPARATION OF A LIPID BLEND AND A PHOSPHOLIPID SUSPENSION
CONTAINING THE LIPID BLEND

5

Field of the Invention

The present invention relates generally to processes for the preparation of a lipid blend and a uniform filterable phospholipid suspension containing the lipid blend, such suspension being useful as an ultrasound contrast agent.

Background of the Invention

Manufacturing of a phospholipid contrast agent can be divided into the following steps: (1) preparation of lipid blend; (2) compounding the bulk solution, which involves the hydration and dispersion of the lipid blend in an essentially aqueous medium to produce a lipid suspension; (3) filtration of the bulk solution through a sterilizing filter(s) to render the suspension free of microbial contaminants; (4) dispensing the sterile suspension into individual vials in a controlled aseptic area; (5) loading the dispensed vials into a lyophilizer chamber to replace the vial headspace gas with perfluoropropane gas (PFP); (6) transferring the sealed vials after gas exchange to an autoclave for terminal sterilization. There are three major obstacles in this process: (1) uniformity of the lipid blend; (2) hydration of the lipid blend; (3) uniformity and particle size of the suspension; and, (4) sterile filtration of the suspension through a sterilizing filter(s).

Phospholipid blends are typically produced by dissolving or suspending the required lipids in an appropriate aqueous or non-aqueous solvent system, and then reducing the volume either by lyophilization or distillation. Ideally, this process produces blended solids with high content uniformity and purity. However, while working well on a small, laboratory scale, this simple approach is frequently problematic upon scale-up to

production-size quantities. Difficulties include: (1) maintaining content uniformity during the solvent removal step (due to differential solubilities); (2) maintaining purity (frequently a problem when water is used due to hydrolytic side-reactions); (3) enhancing purity; (4) minimizing solvent volume; and (5) recovery of the final solids (e.g., it is not practical to scrape solids out of a large reactor).

After manufacture of a lipid blend, final compounding typically involves introduction of the blend into an aqueous medium. Since phospholipids are hydrophobic and are not readily soluble in water, adding phospholipids or a lipid blend directly into an aqueous solution causes the lipid powder to aggregate forming clumps that are very difficult to disperse. Thus, the hydration process cannot be controlled within a reasonable process time. Direct hydration of phospholipids or a lipid blend in an aqueous medium produces a cloudy suspension with particles ranging from 0.6 μm to to 100 μm . Due to relatively large particle size distribution, the suspension cannot be filtered at ambient temperature when the suspension solution temperature is below the gel-to-liquid crystal phase transition temperatures of lipids. The lipids would accumulate in the filters causing a restriction in the flow rate, and in most cases, the filters would be completely blocked shortly after. Further reduction in the suspension particle size cannot be achieved through a conventional batching process, even after extended mixing (e.g., 6 hours) at elevated temperatures (e.g., 40°C to 80°C) with a commonly used marine propeller.

Although filtration at elevated temperatures, i.e., at above the phase transition temperatures of lipids, is possible, a significant amount of larger lipid particles would still be excluded when a normal filtering pressure is used. In turn, concentrations of the sterile filtrate would have variable lipid content from batch to batch depending on how the lipids are initially hydrated which is in turn

determined by the physical characteristics, e.g., morphology, of the starting materials.

The process of directly hydrating the lipids or lipid blend to produce a uniform suspension and filtration of the suspension through a sterilization filter(s) can be difficult and costly to be scaled-up to any reasonable commercial scale, e.g., >20L.

Thus, the presently claimed processes for manufacture of a lipid blend and the subsequent phospholipid suspension are aimed at solving the above issues by providing a practical process that can be easily scaled and adopted to various manufacturing facilities without extensive modification or customization of existing equipment.

Summary of the Invention

Accordingly, one aspect of the present invention is to provide a novel process for preparing a lipid blend.

Another aspect of the present invention is to provide a novel process for preparing a phospholipid suspension from the lipid blend.

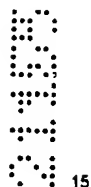
These and other aspects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that dissolving a lipid blend in a suitable non-aqueous solvent prior to introduction of an aqueous solution allows for production of a phospholipid suspension.



Detailed Description of the Invention

Thus, in a first embodiment, the present invention provides a process for preparing a phospholipid suspension, comprising:

- (a) contacting at least two lipids with a first non-aqueous solvent to form
5 a solution;
- (b) concentrating the solution to a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
- (d) concentrating the solution of step (c) to form a lipid blend;
- 10 (e) contacting the lipid blend with a third non-aqueous solvent, whereby the lipid blend substantially dissolves in the third non-aqueous solvent to form a solution; and,
- (f) without removing the third non-aqueous solvent, contacting the solution from step (e) with an aqueous solution to form a phospholipid suspension.



15

In a preferred embodiment, the non-aqueous solvent of step (e) is selected from the group consisting of propylene glycol, ethylene glycol, and polyethylene glycol 300.



20

In a more preferred embodiment, the non-aqueous solvent of step (e) is propylene glycol.

In another preferred embodiment, the lipid blend, comprises:

- (a) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;
- (b) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic, mono sodium salt; and,
- (c) *N*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-
25 glycero-3-phosphatidylethanolamine, mono sodium salt.



In another preferred embodiment, in step (1), the non-aqueous solvent of step (e) is heated to a temperature of about 30 to 70°C prior to contacting with the lipid blend.

5 In another more preferred embodiment, the non-aqueous solvent of step (e) is heated to a temperature of about 50 to 55°C prior to contacting with the lipid blend.

In another preferred embodiment, the ratio of lipid blend to non-aqueous solvent of step (e) is from about 5 mg of lipid blend per mL of non-aqueous solvent
10 to about 15 mg/mL.

In another more preferred embodiment, the ratio of lipid blend to non-aqueous solvent of step (e) is about 10 mg/mL

In another preferred embodiment, in step (f), the aqueous solution is selected from the group consisting of water, saline, a saline/glycerin mixture, and a
15 saline/glycerin/non-aqueous solvent mixture.

In another more preferred embodiment, the aqueous solution is a saline and glycerin mixture.

In another more preferred embodiment, the aqueous solution is a saline, glycerin, and propylene glycol mixture.

20 In another more preferred embodiment, 6.8 mg/mL of sodium chloride are present, 0.1 mL/mL of glycerin are present, 0.1 mL/mL of propylene glycol are present, and about 0.75 to 1.0 mg/mL of the lipid blend are present.

In an even more preferred embodiment, 0.75 mg/mL of lipid blend are present.



In another more preferred embodiment, 1.0 mg/mL of lipid blend are present.

In another preferred embodiment, in step (f), the aqueous solution is heated to a temperature of about 45 to 60°C prior to contacting with the solution from step
5 (e).

In another more preferred embodiment, the aqueous solution is heated to a temperature of about 50 to 55°C prior to contacting with the solution from step (e).

In another preferred embodiment, the process further comprises:

(g) heating the lipid suspension from step (f) to a temperature about
10 equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the suspension.

15

In another more preferred embodiment, in step (g), the lipid suspension is heated to a temperature of at least about 67°C.

20

In another more preferred embodiment, the process further comprises:

(h) filtering the lipid suspension through a sterilizing filter.

In another even more preferred embodiment, in step (h), the filtration is performed using two sterilizing filter cartridges.

In a further preferred embodiment, in step (h), the sterilizing filter cartridges are at a temperature of from about 70 to 80°C.

In another further preferred embodiment, in step (h), 0.2µm hydrophilic filters are used.

In another even more preferred embodiment, the process further
25 comprises:



(i) dispensing the filtered solution from step (h) into a vial.

In another further preferred embodiment, the process further comprises:

(j) exchanging the headspace gas of the vial from step (j) with a perfluorocarbon gas.

5

In another even further preferred embodiment, the perfluorocarbon gas is perfluoropropane.

In another even further preferred embodiment, exchange of headspace gas is performed using a lyophilizing chamber.

10 In another even further preferred embodiment, the process further comprises:

(k) sterilizing the vial from step (j).



15 In a still further preferred embodiment, in step (k), the vial is sterilized at about 126-130°C for 1 to 10 minutes.



In another preferred embodiment, in step (a), the first non-aqueous solvent is a mixture of methanol and toluene.

In another preferred embodiment, in step (c), the second non-aqueous solvent is a methyl *t*-butyl ether.

20 In another preferred embodiment, in step (a), the solution is warmed to a temperature sufficient to complete dissolution of the lipids into the solvent.

In another more preferred embodiment, in step (a), the solution is warmed to about 25 to 75°C.

In another preferred embodiment, in step (d), the solids collected are
25 washed with methyl *t*-butyl ether and dried *in vacuo*.



In a second embodiment, the present invention provides a phospholipid suspension whenever prepared by the above process.

In a third embodiment, the present invention provides a process for preparing a phospholipid blend, comprising:

- 5 (a) contacting at least two phospholipids with a first non-aqueous solvent to form a solution;
- (b) concentrating the solution to a thick gel;
- (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
- 10 (d) concentrating the solution of step (c) to solid phospholipids; and,
- (e) collecting the solid lipids;

wherein the first non-aqueous solvent is a mixture of methanol and toluene; and wherein the second non-aqueous solvent is methyl t-butyl ether.

15 In a fourth embodiment the present invention provides a phospholipid blend whenever prepared by the process as described above.

Formulation

20 The present invention is contemplated to be practiced on at least a multigram scale, kilogram scale, multikilogram scale, or industrial scale. Multigram scale, as used herein, is preferably the scale wherein at least one starting material is present in 10 grams or more, more preferably at least 50 grams or more, even more preferably at least 100 grams or more. Multikilogram scale, as used herein,

25 is intended to mean the scale wherein more than one kilogram of at least one



starting material is used. Industrial scale as used herein is intended to mean a scale which is other than a laboratory scale and which is sufficient to supply product sufficient for either clinical tests or distribution to consumers.

Lipid blend or phospholipid blend, as used herein, is intended to represent
5 two or more lipids which have been blended. The lipid blend is generally in a powder form. Preferably, at least one of the lipids is a phospholipid. Preferably, the lipid blend contains 1,2-dipalmitoyl-*sn*-

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217

glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidic, mono sodium salt (DPPA), and N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt (MPEG5000-DPPE). The amount of each lipid present in the blend will depend on the desired end product. Preferred ratios of each lipid are described in the Examples section. A wide variety of other lipids, like those described in Unger et al. U.S. Patent No. 5,469,854, the contents of which are hereby incorporated by reference, may be used in the present process.

Phospholipid, as used herein, is a fatty substance containing an oily (hydrophobic) hydrocarbon chain(s) with a polar (hydrophilic) phosphoric head group. Phospholipids are amphiphilic. They spontaneously form boundaries and closed vesicles in aqueous media. Phospholipids constitute about 50% of the mass of animal cell plasma membrane.

Preparation of the lipid blend

The lipid blend may be prepared via an aqueous suspension-lyophilization process or an organic solvent dissolution-precipitation process using organic solvents. In the aqueous suspension-lyophilization process, the desired lipids are suspended in water at an elevated temperature and then concentrated by lyophilization. Preferably a dissolution procedure is used.

Step (a):

The organic solvent dissolution-precipitation procedure involves contacting the desired lipids (e.g., DPPA, DPPC, and MPEG5000 DPPE) with a first non-aqueous solvent system. This system is typically a combination of solvents, for example $\text{CHCl}_3/\text{MeOH}$, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, and toluene/ MeOH . Preferably, the first non-aqueous solvent is a mixture of toluene and methanol. It may be desirable to warm the lipid solution to a temperature sufficient to achieve complete dissolution. Such a temperature is preferably about 25 to 75°C, more preferably about 35 to 65°C.

After dissolution, it may be desired to remove undissolved foreign matter by hot-filtration or cooling to room temperature and then filtering. Known methods of filtration may be used (e.g., gravity filtration, vacuum
5 filtration, or pressure filtration).

Step (b):

The solution is then concentrated to a thick gel/semisolid. Concentration is preferably done by vacuum
10 distillation. Other methods of concentrating the solution, such as rotary evaporation, may also be used. The temperature of this step is preferably about 20 to 60°C, more preferably 30 to 50°C.

15 Step (c):

The thick gel/semisolid is then dispersed in a second non-aqueous solvent. The mixture is slurried, preferably near ambient temperature (e.g., 15-30°C). Useful second non-aqueous solvents are those that cause the lipids to
20 precipitate from the filtered solution. The second non-aqueous solvent is preferably methyl *t*-butyl ether (MTBE). Other ethers and alcohols may be used.

Step (d):

25 The solids produced upon addition of the second non-aqueous solvent are then collected. Preferably the collected solids are washed with another portion of the second non-aqueous solvent (e.g., MTBE). Collection may be performed via vacuum filtration or centrifugation,
30 preferably at ambient temperature. After collection, it is preferred that the solids are dried *in vacuo* at a temperature of about 20-60°C.

For the following reasons, the organic solvent
35 dissolution-precipitation process is preferred over the aqueous suspension/lyophilization process:

(1) Because the lipids are quite soluble in toluene/methanol, solvent volumes are significantly reduced (relative to the aqueous procedure).

5 (2) Because of this increased solubility, the process temperature is also lower relative to the aqueous procedure, thereby avoiding the hydrolytic instability of fatty acid esters.

10 (3) When cooled back to room temperature, the toluene/methanol solution of lipids remains homogeneous, allowing a room temperature filtration to remove solid foreign matter.

15 (4) The MTBE precipitation allows quick and easy isolation of Lipid Blend solids. With the aqueous process, a time-consuming lyophilization process is used to isolate material.

20 (5) The MTBE precipitation also allows for the removal of any MTBE-soluble impurities, which go into the filtrate waste-stream. This potential for impurity removal is not realized when a solution is directly concentrated or lyophilized to a solid.

(6) The present process affords uniform solids.

Preparation of the lipid suspension

Step (1):

25 In step one, a lipid blend is contacted with a non-aqueous solvent, whereby the lipid blend substantially dissolves in the non-aqueous solvent. Alternatively, the individual lipids may be contacted with the non-aqueous solvent sequentially in the order: DPPC; DPPA, and
30 MPEG5000-DPPE; DPPC, MPEG5000-DPPE, and DPPA; MPEG5000-DPPE, DPPA, and DPPC; or MPEG5000-DPPE, DPPC, and DPPA. The DPPA, being the least soluble and least abundant of the lipids is not added first. Adding one of the other lipids prior to or
35 concurrently with adding the DPPA facilitates dissolution of the DPPA. In another alternative, the individual lipids can be combined in their solid forms and the combination of the solids contacted with the non-aqueous solvent.

Substantial dissolution is generally indicated when the mixture of lipid blend and non-aqueous solvent becomes clear. As noted previously, phospholipids are generally not water soluble. Thus, direct introduction of a blend of
5 phospholipid blend into an aqueous environment causes the lipid blend to aggregate forming clumps that are very difficult to disperse. The present invention overcomes this limitation by dissolving the lipid blend in a non-aqueous solvent prior to introduction of the aqueous solution. This
10 allows one to evenly disperse the lipid blend into a liquid. The liquid dispersion can then be introduced into a desired aqueous environment.

Non-aqueous is intended to mean a solvent or mixture of solvents wherein the amount of water present is sufficiently
15 low as to not impede dissolution of the lipid blend. The amount of non-aqueous solvent required will depend on the solubility of the lipid blend and also the final desired concentration of each component. As one of ordinary skill would appreciate, the level of water present in the non-
20 aqueous solvent, which may be tolerated will vary based on the water solubilities of the individual lipids in the lipid blend. The more water soluble the individual phospholipids, the more water which may be present in step (1). Preferably, propylene glycol is used as the non-aqueous
25 solvent. However, other members of the polyol family, such as ethylene glycol, and polyethylene glycol 300 may be used.

Mechanically mixing the lipid blend and non-aqueous solvent may be necessary to achieve complete dissolution. One of ordinary skill in the art will recognize that a
30 variety of ways of mixing are available. It is preferred that a high shear homogenizer is used.

One of ordinary skill in the art would recognize that raising the temperature of the solvent should aid in dissolution of the lipid blend. The temperature at which
35 step (1) may be performed can range from ambient to the boiling point of the chosen solvent. Preferably the temperature is from about 30 to about 70°C, more preferably about 45 to about 60°C, and even more preferably about 50.

51, 52, 53, 54, or 55°C. When ethylene glycol or polyethylene glycol 300 is used, it is preferred that the temperature be from about 50 to about 60°C and more preferably about 55°C. Maintaining the solution at an elevated temperature should reduce solution viscosity and ease formulation preparation.

A preferred procedure for dissolving the lipid blend is as follows: (a) Add propylene glycol to an appropriate weighing container. (b) Warm the propylene glycol to about 40-80°C in a heating bath. (c) Weigh the lipid blend into a separate container. (d) When the propylene glycol has reached the desired temperature range, transfer the solution into the container containing the lipid blend. (e) Place the container back into the heating bath until the solution is clear. (f) Mechanically mix the Lipid Blend/Propylene Glycol solution to further assure complete dissolution and uniform dispersion of the lipid blend.

The ratio of lipid blend to non-aqueous solvent will, of course, be limited by the solubility of the lipid blend. This ratio will also be influenced by the desired amount of lipid blend in the final formulation. Preferably, the ratio is from about 1 mg of lipid blend per mL of solvent (mg/mL) to about 100 mg/mL. More preferably, the lipid blend is present in about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mg/mL. Even more preferably, the lipid blend is present in about 10 mg/mL.

Step (2):

The second step involves contacting the solution from step (1) with an aqueous solution to form a lipid suspension. The aqueous solution can be water, saline, a saline/glycerin mixture or a saline/glycerin/non-aqueous solvent mixture. Non-aqueous solvent is as defined previously, preferably propylene glycol. Suspension, as used herein, is intended to indicate a dispersion in which insoluble particles are dispersed in a liquid medium.

Once complete dissolution of the lipid blend has been achieved (step (1)), the resulting solution can then be

introduced to an aqueous solution. The aqueous solution may contain one or more components selected from sodium chloride, glycerin, and a non-aqueous solvent. Preferably the aqueous solution contains glycerin and sodium chloride.

- 5 Preferably, a sufficient amount of propylene glycol is present in the aqueous solution, prior to addition of the solution from step 1, in order to achieve the final desired concentration of propylene glycol.

- The order of addition of desired components is not
10 expected to seriously impact the resulting lipid suspension. However, it is preferred that the lipid-blend solution is added to water, which may already contain the above-noted additional components. Additional desired components can then be added. It is more preferred that the lipid-blend
15 solution is added a solution of water and sodium chloride (i.e., saline). It is further preferred that the lipid-blend solution is added a solution of water, sodium chloride, and glycerin. It is still further preferred that the lipid-blend solution is added a solution of water,
20 sodium chloride, glycerin, and propylene glycol.

- It is preferred that 6.8 mg of NaCl are present per mL of formulation. Preferably, 0.1 mL of Glycerin per mL of formulation is present. A final concentration of 0.1 mL of Propylene Glycol per mL of formulation is preferred. The
25 final pH of the formulation is preferably about 5.5-7.0. The lipid blend is preferably present in an amount of 0.75-1.0 mg/mL of formulation.

- The temperature of the aqueous solution can range from ambient to 70°C. Preferably, the temperature is about 45 to
30 60°C, with 50, 51, 52, 53, 54, or 55 being even more preferred. In order to obtain complete dissolution, the mixture will need to be agitated, preferably stirred. Also, the pH of the solution may need to be adjusted, depending on the desired final formulation. Either acid (e.g., HCl) or
35 base (e.g., NaOH) can be added to make such an adjustment.

The lipid suspension will contain liquid particles of varying sizes. One of the benefits of the present invention is the ability to consistently obtain small particles of a

nearly uniform size. Thus, it is preferred that the majority of particles obtained are less than 100 nm in diameter, more preferable less than 50 nm.

A preferred procedure for dissolving the lipid blend is as follows: (a) Add Water for Injection (WFI) into a compounding vessel. (b) Start mixing and ensure temperature is from 50-55°C. (c) Add sodium chloride to the compounding vessel. Wait until the solid has completely dissolved before proceeding to the next step. (d) Add glycerin to the compounding vessel. Allow sufficient time for complete mixing. (e) Add the remaining Propylene Glycol that is not in the Lipid Blend/Propylene Glycol solution. Allow time for thorough mixing. (f) Reduce mixing rate to reduce turbulence in the compounding vessel. (g) Add the Lipid Blend/Propylene Glycol solution to the compounding vessel. (h) Readjust mixing to original rate. (i) Add additional WFI if necessary. (j) Continue to mix for approximately 25 minutes and assure complete mixing. (k) Verify and adjust the solution to target pH.

20

Step(3):

Step three involves heating the lipid suspension obtained from step (2) to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the solution.

One of the objects of this step is to provide a filterable suspension. A solution/suspension is considered filterable if there is no significant reduction in flow rate within a normal process, and there is no significant increase in the pressure drop in the filtration system.

Experimental data indicates that the lipids in the formulation should be beyond their gel to liquid crystalline phase transition in order to simplify sterile filtration. When the lipids are below the phase transition temperature, the suspension particles are rigid. However, when they are above their respective gel-liquid crystal phase transition temperatures, they are in a more loosely organized configuration and thus, more easily filtered.

DPPE and DPPA show phase transitions of 41°C and 67°C respectively. MPEG5000-DPPE is soluble in water, therefore it does not exhibit a gel-liquid crystal phase transition which is characteristic of most hydrated lipid suspensions.

5 Because the lipids in the preferred formulation all exhibit different gel to liquid phase transitions, the highest phase transition temperature, 67°C, is preferably used to filter the solution. By maintaining temperature at or beyond 67°C, all the lipids are beyond their respective phase transition,

10 assuring the loose configuration while passing through the filters.

Heating may be achieved by jacketing the compounding vessel with a heat exchanging coil. Hot water/steam from a controlled source, e.g., a hot water bath, or a water

15 heater, would deliver sufficient heat to maintain the compounding solution at a set temperature. Other heat sources known to those of skill in the art could also be used.

20 Step (4):

Step four is performed by filtering the lipid suspension through a sterilizing filter. The purpose behind this step being to provide a substantially bacteria-free suspension. A filtrate is considered substantially

25 bacteria-free when the probability of the filtrate to contain at least one colony forming microorganism is less than 10^{-6} .

Filtration is preferably done using sterilizing filter cartridges. Also, a means of forcing the solution through

30 the filters may be required (e.g., pumping or pressurizing). Since the solution being filtered needs to be maintained at a temperature at or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the solution, the filtration should be performed

35 at about this same temperature. In order to accomplish this, the filter (e.g., sterilizing filter cartridges) are preferably enclosed in jacketed filter housings which are continuously heated, e.g., by a hot water stream from a

temperature controlled water bath, to ensure that the suspension is above the lipid phase transition temperatures. The temperature of the sterilizing filter is preferably from 50 to 100°C, more preferably from 60 to 90°C, and even more preferably 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80°C.

One or more sterilizing filters may be used to filter the suspension. The required number will be based on their effectiveness at removing bacteria. It is preferred that two filters are used. The size of the filter pores will be limited by the need to provide a bacteria-free suspension. Preferably, 0.2µm hydrophilic filters are used.

A bulk solution of the preferred formulation was continuously filtered through two 0.2µm hydrophilic filters for up to 3 hours at a rate of approximately 1 liter per minute (1 L/min.), i.e., passing a total of 180 liters of the suspension solution through the filters. The experimental results shows that there is no apparent blockage of filters. Lipid assays indicates that there is no measurable loss during the filtration process (due to accumulation in the filter medium).

A bulk solution of the preferred formulation was compounded at 40°C-80°C, and the suspension was cooled to ambient temperature prior to sterile filtration. No apparent clogging of the filters were observed indicating the suspension particle size distribution is well below 0.2µm of the filter pore size. It is desirable to use heat during filtration in order to ensure maximum recover of the lipid blend in the sterile filtrate (i.e., to minimize potential retention of lipid particles in the filter medium).

A preferred procedure for filtering the lipid suspension is as follows: (a) Assure all jacketed filters are at 70°C - 80°C. (b) Assure all valves in the filtration unit are closed. (c) Connect filtration inlet hose to the outlet of the compounding vessel. (d) Open valves to allow solution to pass through the filters. (e) Flush three liters of solution through the filters before collecting filtrate. (f) Continue filtration until complete.

Step (5):

Dispensing the filtered solution into a vial completes step five. Preferably, this step is performed in a controlled aseptic area. One of ordinary skill in the art would recognize that the vial selected and amount of suspension delivered to the vial would depend on the end use considered for the lipid suspension. Dispensing can be achieved via a variety of methods, including pipette, hand-held syringe dispenser (e.g., Filamatic® syringe dispensing machine), or industrial auto dispensing machine (e.g., Cozzoli or TL auto filling machine).

Step (6):

Step six is performed by exchanging the headspace gas of the vials from step five with a perfluorocarbon gas. A preferred method of exchange is to load the dispensed vials into a lyophilizer chamber and replace the vial headspace gas with a perfluorocarbon gas. A preferred gas is perfluoropropane (PFP). Other methods of headspace gas exchange known to those of skill in the art may be employed.

The vials are sealed at the completion of the vial headspace gas exchange cycle. When the lyophilizer chamber pressure is brought back to atmospheric pressure by charging into the chamber with PFP. Vial stoppers are seated to seal the vials.

Step (7):

Step seven involves terminally sterilizing a vial after step six. One method of terminal sterilization is through the use of an autoclave. Also, the sealed vials can be terminally sterilized in a steam sterilizer to further enhance the sterility assurance of the product. Care must be taken in the sterilization process as some degradation of lipids may be observed as a result of autoclaving. Preferably, the vial is sterilized at about 126-130°C for 1 to 10 minutes.

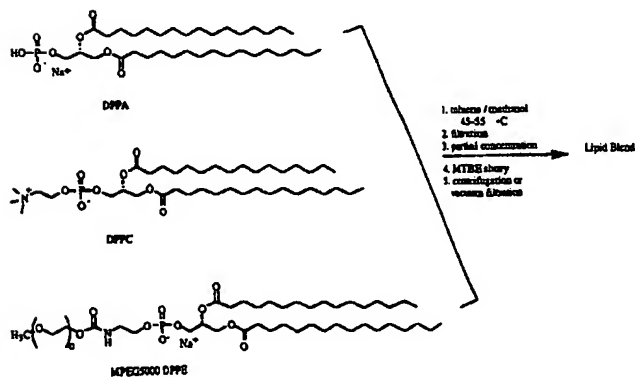
Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

5

EXAMPLES**Table 1: Lipid Blend Target Composition**

Lipid Name	Common Name	Wt. %	Mole %
DPPA	1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, monosodium salt	6.0	10
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine	53.5	82
MPEG5000 DPPE	(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt	40.5	8

10

Lipid Blend Manufacturing Procedure

A flask is charged with toluene (3.3 L), methanol (1.2 L), DPPA (59.6 g), DPPC (535 g), and MPEG5000 DPPE (405 g).

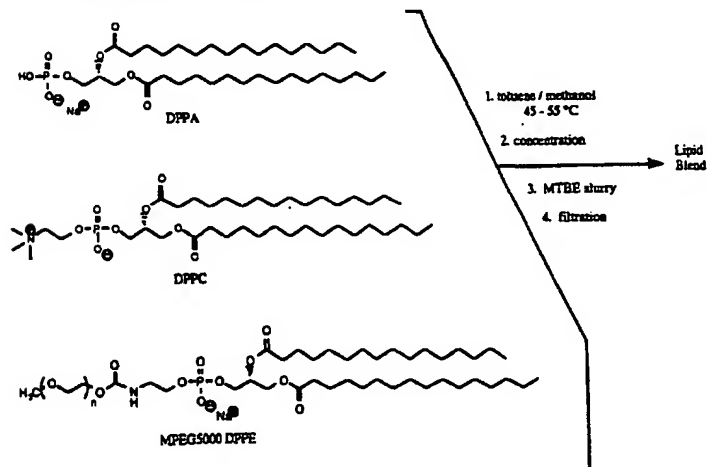
15

After rinsing solid contact surfaces with 0.9 L methanol, the slurry is warmed to 45-55°C until dissolution is complete.

The solution is filtered and then concentrated *in vacuo* at 35 - 45° C to a thick gel. Methyl *t*-butyl ether (MTBE, 5.4 L) is added and the mixture is slurried at 15-30°C. White solids are collected by centrifugation or vacuum filtration, and washed with MTBE (0.9 L). The solids are then placed in a vacuum oven and dried to constant weight at 40-50°C. The dried Lipid Blend is transferred to a bottle and stored at -15 to -25 °C.

In another embodiment of the lipid blend manufacturing procedure of the present invention, the following procedure may also be used.

Alternative Lipid Blend Manufacturing Procedure



20

Phospholipid quantities were adjusted for purity based on a "Use As" value from the certificates of analysis. The

batch size (combined phospholipid weight) of this experiment was 2 kg.

A rotary evaporation flask is charged sequentially with toluene (3,300 mL), methanol (1,200 mL), DPPA (122.9 g; corrected for "use as" purity of 97.0 %), DPPC (1,098.5 g total; 500.8 g from a lot with 98.4 % "use as" purity and 597.7 g from a lot with 96.7 % "use as" purity), and MPEG5000 DPPE (815.7 g; corrected for "use as" purity of 99.3 %). After rinsing residual solids into the flask with methanol (900 mL), the flask is placed on a rotary evaporator (no vacuum) and the slurry is warmed to between 45 and 55 °C (external). After dissolution is complete, the external temperature is reduced to between 35 and 45 °C, a vacuum is applied, and the solution is concentrated to a white semi-solid. The flask is removed from the evaporator and solids are broken up with a spatula. The flask is reapplied to the evaporator and concentration is continued. After reaching the endpoint (final vacuum pressure \approx 20 mbar; white, granular, chunky solid), MTBE (5,400 mL) is added through the rotary evaporator's addition tube, the vacuum is discontinued, and the mixture is slurried for 15 to 45 min at 15 to 30 °C. Solids are isolated by either centrifugal or vacuum filtration, rinsed with MTBE (3,800 mL), and dried to constant weight in a vacuum oven (40 to 50 °C). Prior to transferring to polyethylene bottles with polypropylene caps, solids are delumped through a screen (0.079 inch mesh), affording 1,966.7 g (98 %) of lipid blend (SG896) as a white solid.

The preferred lipid suspension contains:

- 1,2-dipalmitoyl-sn-glycero-3-phosphatidic, mono sodium salt (DPPA);
- 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC);
- N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-

- phosphatidylethanolamine, monosodium salt
(MPEG5000-DPPE);
Propylene Glycol, USP;
Glycerin, USP;
Sodium Chloride, USP; and,
Water for Injection, USP.

Table 2: Preferred Contrast Agent Formulations

Component	A*	B*
NaCl, USP	6.8 mg/mL	6.8 mg/mL
Glycerin, USP	0.1 mL/mL	0.1 mL/mL
Propylene Glycol, USP	0.1 mL/mL	0.1 mL/mL
Lipid Blend**	1 mg/mL	0.75 mg/mL
Perfluoropropane	> 65%	> 65%
pH	6.0 - 7.0	6.0 - 7.0

- *Formulation A has 1 mg/mL lipid blend. Formulation B has a lipid blend concentration of 0.75 mg/mL.
**The lipid blend is consist of 53.5 wt.% of DPPC, 6.0 wt.% of DPPA and 40.5 wt.% of MPEG5000-DPPE.

Table 3: Preferred Container and Closure

Component	Type
Vial	Wheaton 2802, B33BA, 2cc, 13mm, Type I, flint tubing vial
Stopper	West V50 4416/50, 13mm, gray butyl lyo, siliconized stoppers
Seal	West 3766, white 13mm, flip-off aluminum seals

The finished product fill volume can be from 1.0-2.0 mL/vial.

- In the preparation of the preferred formulation, when the lipid blend is directly hydrated with the aqueous matrix solution containing water for injection, sodium chloride.

glycerin and propylene glycol, the filtrates have less lipids as compared to the pre-filtration bulk solution. The loss of lipids varies from 12% to 48%. These results demonstrate that the sterile filtration process is not effectively controlled, and therefore, the final product lipid content is highly variable.

In contrast, using the presently described process, assay results of the lipids in show full recovery of lipids during the filtration process. Variability of assay results around the theoretical targets is within normal assay method variability. Particle size distribution by number, by volume and by reflective intensity of a suspension prepared by first solubilizing lipid blend in propylene glycol indicate that the majority of the particles are less than 50 nm in the pre-filtered bulk solution at 55°C as well at 70°C. The particle distribution profile does not change after filtration.

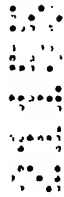
UTILITY SECTION

The presently claimed process is useful for preparing ultrasound contrast agents. Such agents should be useful for a variety of imaging applications, including enhancing contrast in echocardiographic and radiologic ultrasound images.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

30

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification, they are to be interpreted as specifying the presence of the stated features, integers, steps or components referred to, but not to preclude the presence or addition of one or more other feature, integer, step,
5 component or group thereof.



The claims defining the invention are as follows:

1. A process for preparing a phospholipid suspension, comprising:
 - (a) contacting at least two lipids with a first non-aqueous solvent to form a solution;
 - 5 (b) concentrating the solution to a thick gel;
 - (c) contacting the thick gel with a second non-aqueous solvent to form a solution;
 - (d) concentrating the solution of step (c) to form a lipid blend;
 - (e) contacting the lipid blend with a third non-aqueous solvent, whereby
 - 10 the lipid blend substantially dissolves in the third non-aqueous solvent to form a solution; and,
 - (f) without removing the third non-aqueous solvent, contacting the solution from step (e) with an aqueous solution to form a phospholipid suspension.
2. A process according to claim 1, wherein the non-aqueous solvent of
- 15 step (e) is selected from the group consisting of propylene glycol, ethylene glycol, and polyethylene glycol 300.
3. A process according to claim 2, wherein the non-aqueous solvent of step (e) is propylene glycol.
4. A process according to any one of claims 1 to 3, wherein the lipid
- 20 blend comprises:
 - (a) 1, 2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;
 - (b) 1, 2- dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt; and
 - (c) N-(methoxypolyethylene glycol 5000 carbamoyl) -1, 2- dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, mono sodium salt.
- 25 5. A process according to claim 2, wherein the non-aqueous solvent of step (e) is heated to a temperature of about 30 to 70°C prior to contacting with the lipid blend.
6. A process according to claim 5, wherein the non-aqueous solvent of step (e) is heated to a temperature of about 50 to 55°C prior to contacting with the
- 30 lipid blend.
7. A process according to claim 2, wherein the ratio of lipid blend to non-aqueous solvent of step (e) is from about 5 mg of lipid blend per mL of non-aqueous solvent to about 15 mg/mL.



8. A process according to claim 7, wherein the ratio of lipid blend to non-aqueous solvent of step (e) is about 10 mg/mL.

9. A process according to any one of claims 1 to 8, wherein in step (f), the aqueous solution is selected from the group consisting of water, saline, a saline/glycerin mixture, and a saline/glycerin/non-aqueous solvent mixture.

10. A process according to claim 9, wherein the aqueous solution is a saline and glycerin mixture.

11. A process according to claim 9, wherein the aqueous solution is a saline, glycerine, and propylene glycol mixture.

12. A process according to claim 11, wherein 6.8 mg/mL of sodium chloride are present, 0.1 mL/mL of glycerin are present, 0.1 mL/mL of propylene glycol are present, and about 0.75 to 1.0 mg/mL of the lipid blend are present.

13. A process according to claim 12, wherein 0.75 mg/mL of lipid blend are present.

14. A process according to claim 12, wherein 1.0 mg/mL of lipid blend are present.

15. A process according to any one of claims 1 to 4, wherein in step (f), the aqueous solution is heated to a temperature of about 45 to 60°C prior to contacting with the solution from step (e).

16. A process according to claim 15, wherein the aqueous solution is heated to a temperature of about 50 to 55°C prior to contacting with the solution from step (e).

17. A process according to any one of claims 1 to 16, wherein the process further comprises:

(g) heating the lipid suspension from step (f) to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the suspension.

18. A process according to claim 17, wherein in step (g), the lipid suspension is heated to a temperature of at least about 67°C.

19. A process according to claim 17, wherein the process further comprises:

(h) filtering the lipid suspension through a sterilizing filter.

20. A process according to claim 19, wherein in step (h), the filtration is performed using two sterilizing filter cartridges.



21. A process according to claim 20, wherein in step (h), the sterilizing filter cartridges are at a temperature of from about 70 to 80°C.

22. A process according to claim 21, wherein in step (h), 0.2µm hydrophilic filters are used.

5 23. A process according to claim 19, wherein the process further comprises:

(i) dispensing the filtered solution from step (h) into a vial.

24. A process according to claim 23, wherein the process further comprises:

10 (j) exchanging the headspace gas of the vial from step (i) with a perfluorocarbon gas

25. A process according to claim 24, wherein the perfluorocarbon gas is perfluoropropane.

15 26. A process according to claim 25, wherein exchange of headspace gas is performed using a lyophilizing chamber.

27. A process according to claim 24, wherein the process further comprises:

(k) sterilizing the vial from step (j).

20 28. A process according to claim 27, wherein in step (k), the vial is sterilized at about 126-130°C for 1 to 10 minutes.

29. A process according to any one of claims 1 to 28, wherein in step (a), the first non-aqueous solvent is a mixture of methanol and toluene.

30. A process according to any one of claims 1 to 29, wherein in step (c), the second non-aqueous solvent is a methyl *t*-butyl ether,

25 31. A process according to any one of claims 1 to 30, wherein in step (a), the solution is warmed to a temperature sufficient to complete dissolution of the lipids into the solvent.

32. A process according to claim 31, wherein in step (a), the solution is warmed to about 25 to 75°C.

30 33. A process according to any one of claims 1 to 32, wherein in step (d), the solids collected are washed with methyl *t*-butyl ether and dried in *vacuo*.

34. A process for preparing a phospholipid blend, comprising:

(a) contacting at least two phospholipids with a first non-aqueous solvent to form a solution;



(b) concentrating the solution to a thick gel;
(c) contacting the thick gel with a second non-aqueous solvent to form a solution;

(d) concentrating the solution of step (c) to solid phospholipids; and,
5 (e) collecting the solid lipids;

wherein the first non-aqueous solvent is a mixture of methanol and toluene; and wherein the second non-aqueous solvent is methyl t-butyl ether.

35. A process of any one of claims 1 to 33 for preparing a phospholipid suspension which process is substantially as herein described with reference to
10 any one of the Examples.

36. A phospholipid suspension whenever prepared by the process of any one of claims 1 to 33 or claim 35.

37. A process of claim 34 for preparing a phospholipid blend which process is substantially as herein described with reference to any one of the
15 Examples.

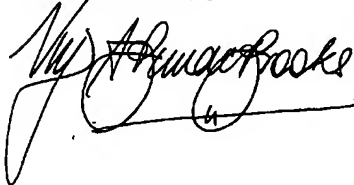
38. A phospholipid blend whenever prepared by the process of claim 34 or claim 37.

20 Dated this 6th day of February, 2002.

DU PONT PHARMACEUTICALS COMPANY

By their Patent Attorneys:

CALLINAN LAWRIE



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.